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Genetic diversity and relationships detected by ISSR and RAPD analysis among *Aethionema* species growing in Eastern Anatolia (Turkey)

Serap Sunar [a,*], Nalan Yildirim [b], Meryem Sengul [c], Guleray Agar [c]

[a] Department of Pharmaceutical Botany, Faculty of Pharmacy, Erzincan University, 24000 Erzincan, Turkey
[b] Department of Biology, Faculty of Science, Erzincan University, 24000 Erzincan, Turkey
[c] Department of Biology, Faculty of Science, Atatürk University, 25240 Erzurum, Turkey

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ABSTRACT

In this study, Random amplified polymorphic DNA (RAPD) and Inter-Simple Sequence Repeat (ISSR) analysis were used to examine the genetic relationships among eight *Aethionema* species (*Aethionema caespitosum*, *A. arabicum*, *A. cordatum*, *A. fimnraitum*, *A. armenum*, *A. speciosum* supsp. *speciosum*, *A. memraneceum*, *A. grandiflorum* var. *grandiflorum*) growing in the wild in Eastern Anatolia, Turkey. Fourteen RAPD primers and 7 ISSR primers were used. The UPGMA cluster was constructed using a combination of data from RAPD and ISSR markers. The *Aethionema* species were classified into two major groups. The similarity matrix values of between 0.182 (*A. cordatum*, *A. speciosum* supsp. *speciosum*) and 0.927 (*A. grandiflorum* var. *grandiflorum*, *A. cordatum*). High genetic variations among *Aethionema* species growing in the wild in Eastern Anatolia, Turkey may reveal differences in their origin. The present study suggests that both RAPD and ISSR analysis are useful for the differentiation of the *Aethionema* species.

1. Introduction

Aethionema R. Br. (Brassicaceae) is a taxonomically complex genus, and a few macromorphological characters are available for species delamination. *Aethionema* is a genus of flowering plants, within the family Brassicaceae, subfamily Brassicoideae. They originate from limestone mountainsides in Europe and Western Asia, especially Turkey. Life duration (annular/perennial) and fruit morphology are of impotence at the species level in *Aethionema*. The genus has its center in Turkey, and outside Anatolia its population declines very rapidly [1]. The Turkish flora is comprised of about 41 *Aethionema* species, of which 20 species are endemic to Turkey [2–4].

Most species of the Brassicaceae develop fruits in which seeds are released through a process termed fruit dehiscence. Some genera in Brassicaceae develop indehiscent fruits that do not release ripe seeds. The genus *Aethionema* is the sister group to all other extant Brassiceceae, some species of *Aethionema* are heterocarpic, meaning that they develop as both dehiscent and indehiscent.

Earlier classifications and evaluations of the genus *Aethionema* were done primarily based on phenotypic expressions of the plants such as growth form, leaf morphology, fruit properties such as color, length of the styles in female flowers and other agronomical characters, but information from these environmentally influenced morphological and physiological characteristics are not

* Corresponding author.
E-mail address: ssunar@erzincan.edu.tr (S. Sunar).

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sufficient to identify *Aethionema* because the differences between them are often subtle and misleading. Hence, recent advances in the field of molecular biology and gene technology have been successfully used for the evaluation of the genetic relationship between plants species in addition to their morphological characters [5–10]. The RAPD and ISSR techniques, when compared to AFLP and SSR, are fast and easy, since they do not require knowledge of the sequences of the markers and can produce abundant polymorphic fragments. So far, there have been few attempts to study the genetic variation in *Aethionema species* using molecular analyses [11,12]. However, no studies have been conducted to assess the genotypic differences in most of *Aethionema species*, including those encountered in Eastern Anatolia (Turkey). Therefore, this study aimed at determining the genetic relationship among *Aethionema caespitosum, Aethionema arabicum*, *Aethionema cordatum*, *Aethionema fimnraitum*, *Aethionema armenum*, *Aethionema speciosum* supsp. *speciosum*, *Aethionema memraneceum*, *Aethionema grandiflorum* var. *grandiflorum species* growing in Eastern Anatolia using RAPD and ISSR technologies. The information obtained will be useful in the genetic analysis of *Aethionema species.*

2. Materials and methods

2.1. Plant material

A sample collection of eight species of *Aethionema* (*A. caespitosum*, *A. arabicum*, *A. cordatum*, *A. fimnraitum*, *A. armenum*, *A. speciosum* supsp. *speciosum*, *A. memraneceum*, *A. grandiflorum* var. *grandiflorum*) was collected at the flowering stage from different locations in the vicinity of Erzurum, Bayburt, located in Eastern Anatolia, Turkey (Table 1). Plant materials were ground in a grinder equipped with a 2-mm diameter mesh. The powdered plant material was then used for DNA extraction. The voucher specimen has been deposited at the herbarium, Department of Biology, Atatürk University, Erzurum, Turkey (Table 1). Plants were collected around Erzurum in 2011–2012 and deposited at ATA (Atatürk University Herbarium).

2.2. DNA extraction

Genomic DNA was extracted from powdered plant materials using a method described by Sunar et al. [13]. The purity and quantity of genomic DNA was determined spectrophotometrically and confirmed using 0.8% agarose gel electrophoresis against known concentrations of unrestricted lambda DNA.

2.3. RAPD amplification

Forty-five primers had been attempted to generate RAPD profiles. Fourteen of these primers were selected: they produced amplicons with all of the *Aethionema* species tested, which were used in further studies based on the results of the preliminary tests (Table 2). PCR amplification reactions were carried out in a final reaction mixture volume of 30 μl, containing 10 × Buffer 3.0 μl, dNTPs (10 mM) 1.2 μl, magnesium chloride (25 mM) 1.2 μl, primer (5 μM) 2.0 μl, Taq DNA polymerase (5unit) 0.4 μl, water 19.2 μl sample DNA 3.0 μl (100 ng/μl). The thermal cycler (Eppendorf Company) was programmed as 2 min at 95 °C; 2 cycles of 30 s at 95 °C, 1 min at 37 °C, 2 min at 72 °C; 2 cycles of 30 s at 95 °C, 1 min at 35 °C, 2 min at 72 °C; 41 cycles of 30 s at 94 °C, 1 min at 35 °C, 2 min at 72 °C; followed by a final 5-min extension at 72 °C, then brought down to 4 °C.

2.4. ISSR amplification

A total of 34 ISSR primers were tested for DNA amplification. Seven primers were chosen for ISSR analyses of genetic diversity, based on band reproducibly (Table 2). PCR reactions were carried out using a single primer at a time, in a 25-mL reaction mixture containing 40 ng of template DNA, 1 × reaction buffer, 200 mM of each of the four dNTPs, 1 U of Taq DNA polymerase, 1.5 mM of magnesium chloride and 0.5 mM of primer. Amplification was performed using a thermal cycler programmed for an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 45 s at 94 °C, 1 min at the specific annealing temperature and 1 min at 72 °C, ending with a final extension step of 7 min at 72 °C. The PCR products of ISSR markers were resolved by electrophoresis on 1.5% agarose gels.

2.5. Electrophoresis

The PCR products (27 μl) were mixed with a 6 × gel loading buffer (3 μl) and submitted to agarose (1.5% w/v) gel electrophoresis in a 0.5XTBE (Tris-Borate- EDTA) buffer

Table 1
Aethionema species tested in this study.

Aethionema species tested in this study	Herbarium number of the Vouchers	Locality	Status	Altitude
Aethionema caespitosum (Boiss.) Boiss.	9848	Kop mountain	End	2100
Aethionema arabicum (L.) Andrz. Ex DC	9849	Erzurum	–	2250
Aethionema cordatum (Desf.)	9850	Kop mountain	–	2180
Aethionema fimnraitum Boiss.	9851	Erzurum Hınıs	Ir–Tur	2200
Aethionema armenum Boiss.	9852	Erzurum Hınıs	Ir–Tur	2350
Aethionema speciosum Boiss. & A. Huet	9853	Kop mountain	Ir–Tur	2200
Aethionema memraneceum (Desu.) DC	9854	Erzurum Hınıs	–	2300
Aethionema grandiflorum Boiss. & Halenvar. *grandiflorum*	9855	Erzurum Tekman	–	2100